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# ผลของการเหนี่ยวนำให้เกิดการติดเชื้อ *Aeromonas hydrophila* ต่อดัชนีชี้วัดความเครียดในปลาดุกบิ๊กอุย (*Clarias gariepinus x C. macrocephalus*)

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**บทคัดย่อ**: ปลาดุกบิ๊กอุย (*Clarias gariepinus* x *C. macrocephalus*) น้ำหนัก 11.4±2.8 กรัม มีความยาวจากปลายปากถึงปลายหาง 12.4±0.9 เซนติเมตร นำมาแบ่งออกเป็น 2 กลุ่ม คือ กลุ่มแรกเหนี่ยวนำให้ปลาดุกเกิดการติดเชื้อ *Aeromonas hydrophila* โดยการฉีด *A. hydrophila* เข้าช่องท้อง ในระดับที่ทำให้เกิดความเครียดเฉียบพลัน (1 วัน) และกลุ่มที่ 2 เหนี่ยวนำให้ปลาดุกเกิดการติดเชื้อ *A. hydrophila* ในระดับที่ทำให้เกิดความเครียดเรื้อรัง (4 วัน) ปลาดุกทุกตัวถูกวางยาสลบก่อนฉีดเชื้อ *A. hydrophila* เข้าช่องท้องและเจาะเลือด หลังจากนั้นสุ่มปลาทั้งสองกลุ่มเพื่อเจาะเลือดจาก caudal vein โดยปลากลุ่มแรกเจาะเลือดที่เวลา 0, 3, 6 และ 24 ชั่วโมง และปลากลุ่มที่ 2 เจาะเลือดในวันที่ 1, 2, 3 และวันที่ 4 นำเลือดที่ได้ทั้งหมดตรวจหาดัชนีชี้วัดความเครียดดังนี้ คือ เวลาการแข็งตัวของเลือด (whole blood clotting time) คอร์ติซอล (cortisol) กลูโคส (glucose) ออสโมลาริตี้ (osmolarity) และการตรวจหาระดับสารสื่อนำไฟฟ้าในเลือด (plasma electrolytes ได้แก่ $Na^+$, $K^+$ และ $Cl^-$) ผลการทดลองพบว่า whole blood clotting time และ cortisol จากกลุ่มควบคุมไม่มีความแตกต่างกันทางสถิติ ($P>0.05$) ค่า Osmolarity และค่า glucose ในกลุ่มของการติดเชื้อเฉียบพลันและติดเชื้อเรื้อรังมีความแตกต่างกันทางสถิติ ($P<0.05$) ส่วนค่า plasma electrolytes จากกลุ่มควบคุมมีค่าคงที่ นอกจากนี้จากการทดลองพบปลาในกลุ่มควบคุมไม่มีความเครียด ส่วนปลาที่เหนี่ยวนำให้ติดเชื้อทั้งในระดับที่ทำให้เกิดความเครียดเฉียบพลันและในระดับที่ทำให้เกิดความเครียดเรื้อรัง แม้จะไม่ตาย แต่จะมีค่าของระดับน้ำตาลที่แตกต่างกันคือ ปลาดุกในกลุ่มที่มีการเหนี่ยวนำให้ติดเชื้อในระดับที่ทำให้เกิดความเครียดเฉียบพลันจะพบว่ามีระดับน้ำตาลในเลือดสูง แต่ในทางตรงกันข้าม ปลาดุกในกลุ่มที่มีการเหนี่ยวนำให้ติดเชื้อในระดับที่ทำให้เกิดความเครียดเรื้อรังจะพบว่ามีระดับน้ำตาลในเลือดต่ำ

**คำสำคัญ**: *Aeromonas hydrophila* เลือดปลา สุขภาพปลา ปลาดุกบิ๊กอุย ดัชนีชี้วัดความเครียด

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# Impact of Artificial *Aeromonas hydrophila* Infection on Stress Indicator in Hybrid Catfish (*Clarias gariepinus x C. macrocephalus*)

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**Abstract**: Hybrid catfish (*Clarias gariepinus x C. macrocephalus*), 11.4±2.8 g in body weight and 12.4±0.9 cm in body length, were submitted to stress analysis in a laboratory. This research was composed of two experiment groups. The first group injected *Aeromonas hydrophila* into intraperitoneum for inducing acute stress that was a one-day experiment. Another group induced chronic stress that was a four days experiment, by injecting *A. hydrophila* into intraperitoneum. All catfish were drawn from the caudal vein before and after *A. hydrophila* injection. Fish with acute stress had blood taken at 0, 3, 6 and 24 hr. Chronic stressed fish had blood taken at the 1$^{st}$, 2$^{nd}$, 3$^{rd}$, 4$^{th}$ day. The catfish blood was investigated for stress indicator indices. These were blood clotting time, cortisol, glucose, osmolarity, plasma electrolytes ($Na^+$, $K^+$, and $Cl^-$). Blood clotting time and cortisol from the control group had no significant differences ($P>0.05$). Osmolarity in both the acute and chronic bacterial stressed were significantly different ($P<0.05$). Plasma electrolytes from the control group were stable. Blood glucose from acute and chronic infection indicated statistically significant differences ($P<0.05$). Moreover, the results revealed that the catfish in the control group were not stressed. Infected fish that are induced to acute and chronic *A. hydrophila* infection will have problems with blood sugar. In acute stress caused by *A. hydrophila* infection, the catfish were subjected to hyperglycemia. Hypoglycemia was found in fish when exposed to chronic infection stress.

**Keywords**: *Aeromonas hydrophila*, Fish blood, Fish health, Hybrid catfish, Stress indicator

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## Introduction

Catfish are well adapted and able to compensate for living in a variety of environmental culture systems. Hybrid catfish are widely chosen to be raised because of their fast growth rate and high diseases resistance

(Koeypudsa et al, 2006). Changes in the environmental fish culture system, both biotic and abiotic factors, constitute stress in fish (Small, 2004). Abiotic stressors are culture management, physico-chemical water quality, nutrition, confinement, capture and handling. Meanwhile, biotic stress factors are predators, plankton, virus, bacteria, parasite, age and gender (Komen et al., 2001; Chen et al., 2003; Chen et al., 2004; Pepels and Balm, 2004; Silva et Al., 2012).

Blood parameters are useful in clinical veterinary practice to monitor and diagnose fish pathologies (Chen et al., 2003; Koeypudsa and Jongjareanjai, 2011). The stress hormone, cortisol is a principal response to stressors and is applied as a primary stress response indicator (Davis, 2004; Guerriero et al., 2002; Lerman et al., 2004). Cortisol performs the important role of homeostasis during and after stress (Pacheco and Santos, 2001; Goos and Consten, 2002). Plasma glucose and plasma electrolytes are secondary responses. Both blood sugar and blood electrolytes are affected by stress hormones, cortisol (Koeypudsa and Jongjareanjai, 2011; Lerman et al., 2004; Ham et al., 2003). Glucose levels can be clinically important to apply since levels can easily vary with regard to both biotic and abiotic stressors. Blood clotting time can be used as a general stress indicator in fish affected by environmental stressor and the presence of bacteria (Koeypudsa et al., 2006). Clotting blood is easy to use for the purpose of stress detection because only a few drops of blood are needed. It is a rapid method and a non-lethal and inexpensive tool (Harikrishnan et al., 2012; Peres et al., 2012).

The objectives of this study were to obtain blood data and evaluate stress in hybrid catfish that had been subjected to *A. hydrophila*. Blood parameters were applied as a stress indicator for acute and chronic bacterial stress, at 1-day and 4-days of observation. The benefit of this study is that it is expected to increase the knowledge and use of blood parameters as a stress indicator. The ultimate goal is to apply blood parameters as a monitor of fish health.

## Materials and Methods

This research was perform under a protocol approved by the Ethics Committee for Animal Experimentation, Faculty of Veterinary Science, Chulalongkorn University, and was followed National Research Council of Thailand protocol.

### Hybrid catfish

Hybrid catfish (*Clarias gariepinus x C. macrocephalus*) with a mean body weight and total length of 11.4±2.8 gm and 12.4±0.9 cm, were purchased from a private farm in Suphanburi province. They were fed with commercial pellet feed every afternoon and their water was 10% changed every morning. All the catfish were acclimated for 15 days and were not fed for 1 day before the experiment started. Each experimental catfish was used only once and surviving fish were raised in a fiberglass tank.

**Experimental design**

The experimental catfish were divided into 2 groups for the 1-day and 4-day experiments. Acute stress was observed in the 1-day and chronic stress was observed in the 4-day experiment. At high water temperature (29.5 ± 0.5$^{O}$C), the catfish were placed in an open-air room. Low water exposures (19.5 ± 0.5$^{O}$C) was conducted in an air-conditioned room. All the experimental catfish were kept in water with continuous aeration. The de-chlorinated water quality from the experimental aquarium had the following characteristics: 5.1-5.5 mg/L dissolved oxygen, 7.1-7.3 pH, 105-108 mg/L alkalinity, 85-93 mg/L hardness, 0-0.2 mg/L ammonia, 0.02-0.03 mg/L nitrite, 0.4-0.6 mg/L nitrate and 6-9 mOsmol/L osmolarity.

**Mean lethal dose (LD50)**

Bacteria were isolated from naturally infected hybrid catfish from Roi-ed Province and were identified as *Aeromonas hydrophila*. Two groups of catfish were raised separately in an air-conditioned room (19.5±0.5$^{O}$C) and an open-air room (29.5±0.5$^{O}$C). In each room, the experimental catfish were grouped into five, four experimental and 1 control, with 10 fish in each. The control group was injected intraperitoneally (IP) with 0.1 mL physiological saline. The four experimental groups were IP with 0.1 mL of *A. hydrophila* containing 2.6 x $10^{2}$ – 5.8 x $10^{12}$ colony forming unit (cfu)/mL. The dosages of bacterial concentration were used to determine the median lethal dose (LD50) at 1-day and 4-day observation. Then the obtained data was calculated by probit analysis for LD30 and LD90.

**Acute stress experiment**

Four hundred catfish were separated into 2 rooms, an air-conditioned (19.5 ± 0.5$^{O}$C) and open-air room (29.5±0.5$^{O}$C). Each room was grouped as follows: control, LD30, LD50 and LD90 group. Each group consisted of 10 fish/60 L glass aquarium in 5 replicates. The fish in the control group were IP with 0.1 mL normal saline water. In the air-conditioned room (19.5±0.5$^{O}$C), every fish from LD30, LD50 and LD90 was IP with *A. hydrophila* containing 1.8 x $10^{4}$, 2.6 x $10^{5}$ and 1.2 x $10^{6}$ cfu/mL respectively. In the open-air room (29.5±0.5$^{O}$C) each fish from LD30, LD50 and LD90 was IP with 2.4 x $10^{5}$, 8.9 x $10^{7}$ and 5.4 x $10^{9}$ cfu/mL respectively. Living fish were randomly selected and sedated to withdraw blood from the caudal vein at 0, 3, 6 and 24 hrs.

**Chronic stress experiment**

Four hundred catfish were divided into 2 rooms, low water temperature (19.5 ± 0.5$^{O}$C) and high water temperature (29.5±0.5$^{O}$C). Each room was grouped as following: control, LD30, LD50 and LD90 group. Each group consisted of 10 fish/60 L glass aquarium in 5 replicates. The fish in the control group were IP with 0.1 mL sterilized saline water. Each fish from the air-conditioned room (19.5±0.5$^{O}$C), LD30, LD50 and LD90 was IP with *A. hydrophila* containing 4.0 x $10^{3}$, 1.9 x $10^{4}$ and 5.4 x $10^{5}$ cfu/mL respectively. Each fish in the open-air room (29.5±0.5$^{O}$C) LD30, LD50 and LD90 was IP with 6.4 x $10^{4}$, 3.1 x $10^{5}$ 9.2 x $10^{7}$ cfu/mL respectively. Living fish were

randomly taken and tranquillized for blood withdrawal from the caudal vein at 1, 2, 3 and 4-days.

**Blood determination**

Blood clotting time was assessed by the glass slide method. Serum cortisol was determined by radioimmuno-assay (Coat-A-Count Cortisol®, Diagnostic Products Corporation, USA). Plasma glucose was investigated with an Automated Clinical Chemistry Analyzer (Sapphire 350®, Audit Diagnostics Ltd., Carrigtwohil, Ireland). Plasma osmolarity was obtained with a Cryoscopic Osmometer (Osmomat 030, Gonotec, Berlin). Plasma $Na^+$, $K^+$, and $Cl^-$ were measured using Vitros DTEII module (Johnson & Johnson Clinical Diagnostics, Careside Inc., CA).

**Statistical Analysis**

Obtained data in each experiment was examined for significant differences by one-way analysis of variance following Duncan's multiple range test. Significance was accepted when $P<0.05$. Values were presented as means ± SD.

## Results and Discussion

Blood parameters are shown and expressed as stress indicators. The primary stress indicator is cortisol and the secondary stress indices are glucose, osmolarity and plasma electrolytes: $Na^+$, $K^+$, and $Cl^-$. Moreover, blood clotting time is expressed as stress in general. Acute stress in the 1-day experiment, caused by *A. hydrophila*, is shown in Tables, 1 and 2, when the catfish were raised in low and high water temperatures. Chronic stress in the 4-day observation, caused by bacterial infection, is shown in Tables, 3 and 4, when the fish were kept in a low and high water temperature. Moreover, there is statistical significance among treatments under the same temperature.

**Blood clotting time**

The present study showed that there was no difference of blood clotting time in control group. There was a 44.20–111.40 second clotting time in range. Blood clotting time is an indicator of stress in crustaceans and fish. The prolongation of time was reported in *A. hydrophila* infected fish and those with anoxic exposure (Koeypudsa et al., 2006; Koeypudsa and jongjareanjai, 2011).

This research demonstrates that blood clotting time from chronic infection was statistically increased, whereas blood clotting time in the acute group was unstable. This may indicate that fish from the chronic group were being stressed and fish in the acute group were still attempting to maintain their internal balances. This result is the same as Koeypudsa et al. (2006), who found that anoxic catfish had an increased response with an increase blood clotting time.

The blood clotting time was taken from the surviving fish and those that successfully recovered from bacterial infection. This suggested that the blood clotting time would better be taken from moribund rather than recovered fish. Most of all, this single parameter was not enough criteria to judge the effects of

bacterial infection. Not only blood clotting time but bacterial culture should also be done.

**Cortisol**

In the control group, the cortisol of the fish was not significantly different. The cortisol ranges were 25.47–44.86 ng/mL This means that the catfish were not under stress. The present study shows that cortisol in high water temperature was lower than cortisol in low water temperatures. Water temperature appeared to be a major factor influencing cortisol in the control group. The effect of low water temperature is an increase in the rate of cortisol release (Davis 2004; Ham et al., 2003; Koeypudsa and Jongjareanjai, 2011).

In the infected group (LD30, LD50 and LD90), the fish showed variable levels of cortisol. Even though the concentrations of cortisol were various, the concentration in the final experiment was close to the initial level. It may be assumed that catfish try to adjust their mechanisms to cope with bacterial stress. When catfish successfully adapt themselves to stress situations, cortisol is significantly decreased. The results of this study show that cortisol was decreased within 3 hr. of IP and the trend was to be stable. Small (2004) addressed the phenomenon that the plasma cortisol of confined stressed channel catfish becomes significantly increased within 10 minutes and tends to be constant at 50 minutes. Tanck et al., (2000) showed that common carp cortisol rose within 20 minutes of a cold shock and was down to normal within 60 minutes. Davis (2004) reported that sunshine bass cortisol sharply increased for 1 hr and returned to the start level after 2 hr when exposed to confinement stress.

The results from this study may indicate that unstable cortisol from the infected groups might have a positive effect on fish health and that increasing cortisol caused by stress leads to a suppression of immunity in fish (Ndong et al., 2007).

**Glucose**

Blood glucose from the control group was disturbed in all the experiments. This could indicate that the catfish were attempting to tune their internal balance and physiological mechanisms to compensate for environmental conditions (Peres et al., 2012). Koeypudsa and Jongjareanjai (2011) addressed the fact that hybrid catfish took 10 days to adjust themselves from high temperatures (29±0.5°C) to low temperatures (19±0.5°C). In this research, the catfish from the control group took 1 day and 4 days from high to low water temperatures and this observation may contribute to future studies. Acclimation of fish at both high and low water temperatures should be done to reducing glucose variation.

Plasma glucose from acute infection in this study was high. Increasing blood glucose is related to stress induction (Chen et al., 2004; Dabrowski et al., 2004; Koeypudsa and Jongjareanjai, 2011; Lerman et al., 2004; Osman et al., 2010; Roche and Boge, 2000). It may be assumed that fish with acute infection had increasing stress from bacterial infection (Al-

Dohail et al., 2011, Makarsa et al., 2020). In addition to this study, Al-Dohail et al. (2011) reported that the increased glucose levels could be because of the greater energy requirement to support the metabolism. Glycogen in the liver is converted to glucose and is then exported to the blood stream causing bacterial infection (Datta and Kaviraj, 2003; Perez-Rostro et al., 2004; Yu et al., 2010).

In contrast, with chronic stress, blood glucose from chronic infection in this study was low. This decreasing blood glucose is related to stress reduction (Chen et al., 2004; Harikrishnan et al., 2003; Harikrishnan et al., 2010; Koeypudsa and Jongjareanjai, 2011; Roche and Boge, 2000). This result suggests that fish suffer from chronic infection were in a mildly stressed condition and there had been a health improvement (Al-Dohail et al., 2009; Harikrishnan et al., 2010; Rehulka and Minarik, 2007). Das et al. (2011) demonstrated that glucose from *A. hydrophila* infection in olive barb reduced 7 days after infection and normality was regained after 10 days.

Ndong et al. (2007) and Wassink et al. (2020) addressed the notion that increased cortisol levels lead to increased glucose levels. Even though glucose is a result of cortisol release during stress (Affonso et al., 2002; Gollock et al., 2005a; Gollock et al., 2005b; Grutter and Pankhurst, 2000; Ishibashi et al., 2002), the rise of cortisol was not accompanied with glucose increase in this study.

**Osmolarity**

Fish from the control group presented unstable osmolarity. This might imply that catfish have adapted themselves to maintain hydromineral balance, always gain and loss (Koeypudsa and Jongjareanjai, 2011; Abass et al., 2020). The important component of osmotic balances is the exchange rate of $Na^+$ and $Cl^-$ along with water concentration (Tsuzuki et al., 2001). From this study, this may be assumed that catfish in the control group were unstressed and able to be live without suffering. Most importantly, dead fish were not found in any control group of acute and chronic stress.

This study shows that osmolarity in both the chronic and acute stress group was statistically significantly decreased. Changes of water and ion permeability indicated a stress situation because of osmoregulatory collapse. Osmolarity is affected by cortisol (Eckert et al., 2001; Ruane et al., 2001). Elevation of cortisol causes osmolarity induction and increased branchial efflux (Ziskowski et al., 2008). Catfish is hyperosmotic in a water environment (Koeypudsa and Jongjareanjai, 2011). Fish experience an influx of water of about 50% of body weight/hour (Meyer et al., 2002) and also salt depletion. Those must go on process and must deal with constant body fluid osmolarity.

This study showed unstable cortisol from both acute and chronic stress but osmolarity was low. This might indicate a delayed function of cortisol to osmolarity and mild stress in catfish.

**Table 1.** Blood parameter of acute stress in hybrid catfish caused by *A. hydrophila* infection raised in low water temperature *(19.5 ± 0.5°C)*

| Time *(hr)* | Ct20C | LD30 | LD50 | LD90 |
|---|---|---|---|---|
| **Blood clotting time *(s)*** | | | | |
| 0 | 90.80 ± 15.65 [a] | 109.20 ± 22.68 [b] | 90.40 ± 11.37 [a] | 108.2 ± 8.04 [b] |
| 3 | 100.80 ± 7.19 [a] | 105.20 ± 3.34 [ab] | 95.20 ± 18.10 [a] | 84.80 ± 8.98 [a] |
| 6 | 93.60 ± 18.84 [a] | 91.80 ± 8.78 [ab] | 90.80 ± 3.49 [a] | 88.40 ± 13.06 [a] |
| 24 | 92.20 ± 7.15 [a] | 88.20 ± 3.27 [a] | 89.00 ± 2.44 [a] | 89.80 ± 6.57 [a] |
| **Cortisol *(ng/mL)*** | | | | |
| 0 | 89.72 ± 10.23 [a] | 255.27 ± 128.49 [c] | 93.68 ± 13.24 [c] | 99.04 ± 13.61 [b] |
| 3 | 84.48 ± 30.37 [a] | 150.78 ± 9.93 [ab] | 69.07 ± 14.00 [b] | 5.90 ± 4.31 [a] |
| 6 | 98.29 ± 10.29 [a] | 131.74 ± 17.69 [a] | 38.14 ± 14.33 [a] | 18.10 ± 15.57 [a] |
| 24 | 192.32 ± 23.36 [b] | 240.74 ± 64.7 [bc] | 97.71 ± 15.73 [c] | 98.69 ± 26.36 [b] |
| **Glucose *(mg%)*** | | | | |
| 0 | 93.60 ± 2.96 [b] | 91.60 ± 13.50 [b] | 75.60 ± 14.48 [ab] | 89.80 ± 18.83 [ab] |
| 3 | 177.60 ± 32.30 [c] | 102.80 ± 26.26 [b] | 101.60 ± 12.19 [bc] | 76.80 ± 9.41 [a] |
| 6 | 28.00 ± 8.71 [a] | 36.60 ± 18.02 [a] | 62.60 ± 25.62 [a] | 113.80 ± 11.36 [b] |
| 24 | 80.60 ± 21.33 [b] | 178.00 ± 27.95 [c] | 109.20 ± 26.25 [c] | 166.20 ± 30.21 [c] |
| **Osmolarity *(mosmol/L)*** | | | | |
| 0 | 241.60 ± 32.23 [a] | 288.80 ± 13.55 [b] | 149.80 ± 72.59 [a] | 250.60 ± 22.42 [ab] |
| 3 | 270.20 ± 83.29 [a] | 302.80 ± 25.85 [b] | 166.20 ± 68.99 [a] | 187.40 ± 55.13 [a] |
| 6 | 273.60 ± 7.89 [a] | 306.80 ± 19.76 [b] | 246.80 ± 25.45 [b] | 304.20 ± 28.46 [b] |
| 24 | 309.60 ± 15.86 [b] | 255.60 ± 34.45 [a] | 299.80 ± 22.25 [a] | 207.40 ± 94.12 [a] |
| **$Na^+$ *(mEq/L)*** | | | | |
| 0 | 129.18 ± 2.41 [b] | 117.38 ± 22.97 [a] | 129.36 ± 4.57 [b] | 129.10 ± 4.92 [a] |
| 3 | 128.86 ± 0.64 [b] | 140.98 ± 32.67 [a] | 109.10 ± 20.57 [a] | 122.54 ± 8.53 [a] |
| 6 | 124.34 ± 1.75 [a] | 116.30 ± 6.30 [a] | 127.10 ± 10.20 [b] | 130.22 ± 9.78 [a] |
| 24 | 129.78 ± 2.94 [b] | 126.02 ± 1.81 [a] | 128.70 ± 9.77 [b] | 125.88 ± 5.71 [a] |
| **$K^+$ *(mEq/L)*** | | | | |
| 0 | 3.71 ± 0.11 [b] | 3.87 ± 0.98 [a] | 4.21 ± 0.51 [b] | 4.99 ± 1.66 [b] |
| 3 | 2.55 ± 0.23 [a] | 3.19 ± 0.25 [a] | 2.97 ± 0.65 [a] | 2.74 ± 0.82 [a] |
| 6 | 5.02 ± 0.13 [c] | 4.88 ± 0.28 [b] | 3.22 ± 0.82 [a] | 4.09 ± 0.99 [ab] |
| 24 | 2.44 ± 0.78 [a] | 3.26 ± 0.42 [a] | 2.65 ± 0.60 [a] | 2.94 ± 0.83 [a] |
| **$Cl^-$ *(mEq/L)*** | | | | |
| 0 | 99.52 ± 1.46 [a] | 97.94 ± 8.75 [a] | 101.64 ± 4.94 [b] | 97.34 ± 6.97 [a] |
| 3 | 99.56 ± 0.56 [a] | 97.72 ± 4.18 [a] | 87.34 ± 12.59 [a] | 96.26 ± 6.26 [a] |
| 6 | 104.90 ± 0.63 [b] | 93.92 ± 7.93 [a] | 101.12 ± 4.33 [b] | 98.10 ± 2.83 [a] |
| 24 | 100.20 ± 3.86 [a] | 101.52 ± 5.08 [a] | 99.12 ± 2.02 [b] | 97.60 ± 4.22 [a] |

***** Values *(*mean ±SD, 5 replicates*)* in the same column followed by different superscripts indicate significant difference *(*$P<0.05$, ANOVA, Duncan*)*, Ct20C = control fish at low water temperature, LD30 = 30% fish died at 1-day, LD50 = 50% fish died at 1-day, LD90 = 90% fish died at 1-day

**Table 2.** Blood parameter of acute stress in hybrid catfish caused by *A. hydrophila* infection raised in high water temperature *(29.5 ± 0.5°C)*.

| Time *(hr)* | Ct30C | LD30 | LD50 | LD90 |
|---|---|---|---|---|
| **Blood clotting time *(s)*** | | | | |
| 0 | 48.80 ± 14.66 [a] | 68.40 ± 4.72 [a] | 94.80 ± 7.04 [b] | 78.20 ± 5.76 [a] |
| 3 | 44.20 ± 7.19 [a] | 72.40 ± 13.86 [a] | 62.20 ± 29.37 [a] | 87.20± 16.93 [a] |
| 6 | 65.80 ± 23.93 [a] | 115.60 ± 21.98 [b] | 86.60 ± 7.26 [ab] | 104.40 ± 5.02 [b] |
| 24 | 63.60 ± 23.64 [a] | 75.40 ± 22.72 [a] | 89.40 ± 20.54 [b] | 89.40 ± 6.18 [a] |
| **Cortisol *(ng/mL)*** | | | | |
| 0 | 27.66 ± 9.82 [a] | 141.82 ± 21.71 [b] | 228.26 ± 60.71 [b] | 81.33 ± 9.32 [a] |
| 3 | 48.59 ± 25.64 [ab] | 70.22 ± 17.92 [a] | 98.55 ± 35.61 [a] | 177.97 ± 20.30 [c] |
| 6 | 63.55 ± 9.04 [b] | 75.25 ± 13.13 [a] | 120.75 ± 4.57 [a] | 110.75 ± 10.90 [b] |
| 24 | 162.98 ± 35.09 [c] | 93.22 ± 25.46 [a] | 151.86 ± 34.48 [a] | 99.06 ± 27.56 [ab] |
| **Glucose *(mg%)*** | | | | |
| 0 | 55.60 ± 10.23 [b] | 34.60 ± 5.17 [a] | 69.80 ± 15.22 [b] | 73.20 ± 13.95 [ab] |
| 3 | 42.40 ± 9.04 [ab] | 42.40 ± 18.72 [ab] | 78.40 ± 6.73 [b] | 59.40 ± 9.18 [a] |
| 6 | 37.80 ± 15.8 [a] | 47.40 ± 14.77 [ab] | 73.20 ± 20.12 [b] | 88.20 ± 29.77 [b] |
| 24 | 29.60 ± 7.50 [a] | 54.80 ± 11.23 [b] | 36.40 ± 11.71 [a] | 51.60 ± 12.25 [a] |
| **Osmolarity *(mosmol/L)*** | | | | |
| 0 | 274.80 ± 15.44 [ab] | 256.80 ± 6.30 [a] | 265.40 ± 6.46 [a] | 295.40 ± 5.41 [c] |
| 3 | 256.40 ± 5.59 [a] | 242.20 ± 7.59 [a] | 262.20 ± 5.67 [a] | 300.80 ± 8.78 [c] |
| 6 | 309.20 ± 21.06 [c] | 293.20 ± 26.50 [b] | 270.20 ± 8.75 [a] | 284.60 ± 9.39 [b] |
| 24 | 276.20 ± 7.04 [b] | 265.20 ± 19.00 [a] | 288.40 ± 8.61 [b] | 268.60 ± 3.04 [a] |
| **$Na^+$ *(mEq/L)*** | | | | |
| 0 | 127.14 ± 0.23 [a] | 131.88 ± 3.89 [b] | 132.82 ± 6.57 [a] | 123.12 ± 8.51 [a] |
| 3 | 130.22 ± 6.56 [a] | 125.48 ± 5.26 [a] | 136.64 ± 10.22 [a] | 137.90 ± 4.75 [b] |
| 6 | 135.62 ± 16.62 [a] | 129.12 ± 1.40 [ab] | 129.18 ± 4.31 [a] | 130.36 ± 1.16 [ab] |
| 24 | 128.42 ± 0.81 [a] | 129.42 ± 2.34 [ab] | 136.02 ± 3.11 [a] | 125.34 ± 6.18 [a] |
| **$K^+$ *(mEq/L)*** | | | | |
| 0 | 6.32 ± 0.45 [b] | 5.94 ± 0.40 [c] | 5.74 ± 0.43 [a] | 5.14 ± 1.26 [a] |
| 3 | 5.06 ± 0.47 [a] | 4.22 ± 0.34 [a] | 5.50 ± 0.82 [a] | 5.24 ± 0.18 [a] |
| 6 | 5.14 ± 0.75 [a] | 5.42 ± 0.27 [b] | 6.02 ± 0.63 [a] | 5.48 ± 0.31 [a] |
| 24 | 5.04 ± 0.16 [a] | 5.14 ± 0.46 [b] | 5.32 ± 0.50 [a] | 5.26 ± 0.28 [a] |
| **$Cl^-$ *(mEq/L)*** | | | | |
| 0 | 98.36 ± 2.41 [a] | 104.10 ± 10.32 [b] | 101.38 ± 4.28 [a] | 97.26 ± 4.91 [a] |
| 3 | 102.50 ± 6.67 [a] | 90.68 ± 1.58 [a] | 99.84 ± 10.05 [a] | 100.60 ± 4.51 [a] |
| 6 | 102.62 ± 8.95 [a] | 102.30 ± 7.08 [b] | 100.06 ± 3.75 [a] | 100.36 ± 2.27 [a] |
| 24 | 101.24 ± 1.56 [a] | 102.88 ± 1.25 [b] | 107.18 ± 1.78 [a] | 97.32 ± 5.68 [a] |

***** Values *(*mean ±SD, 5 replicates*)* in the same column followed by different superscripts indicate significant difference *(*$P<0.05$, ANOVA, Duncan*)*, Ct30C = control fish at high water temperature, LD30 = 30% fish died at 1-day, LD50 = 50% fish died at 1-day, LD90 = 90% fish died at 1-day

**Table 3.** Blood parameter of chronic stress in hybrid catfish caused by *A. hydrophila* infection raised in low water temperature *(19.5 ± 0.5°C)*.

| Time *(day)* | Ct20C | LD30 | LD50 | LD90 |
|---|---|---|---|---|
| **Blood clotting time *(s)*** | | | | |
| 1 | 90.60 ± 9.18 [a] | 87.20 ± 5.80 [a] | 97.40 ± 9.78 [a] | 103.40 ± 18.22 [a] |
| 2 | 97.40 ± 5.98 [a] | 119.80 ± 20.78 [b] | 98.60 ± 3.20 [a] | 104.20 ± 15.46 [a] |
| 3 | 93.40 ± 6.06 [a] | 97.60 ± 10.73 [a] | 110.80 ± 10.52 [a] | 118.40 ± 7.23 [ab] |
| 4 | 89.60 ± 11.23 [a] | 95.40 ± 19.39 [a] | 103.80 ± 20.29 [a] | 122.80 ± 3.76 [b] |
| **Cortisol *(ng/mL)*** | | | | |
| 1 | 189.62 ± 52.18 [bc] | 149.82 ± 10.47 [a] | 113.70 ± 29.90 [a] | 177.92 ± 13.38 [b] |
| 2 | 121.73 ± 11.34 [a] | 123.22 ± 33.48 [a] | 170.94 ± 54.94 [b] | 124.91 ± 23.11 [a] |
| 3 | 146.53 ± 33.44 [ab] | 163.33 ± 87.07 [a] | 121.58 ± 32.46 [ab] | 132.62 ± 18.71 [a] |
| 4 | 223.58 ± 38.40 [c] | 160.67 ± 16.39 [a] | 133.46 ± 32.17 [ab] | 140.28 ± 36.40 [a] |
| **Glucose *(mg%)*** | | | | |
| 1 | 21.00 ± 3.39 [a] | 22.40 ± 9.39 [ab] | 24.80 ± 8.70 [bc] | 9.40 ± 6.80 [a] |
| 2 | 23.20 ± 7.01 [a] | 39.40 ± 13.18 [c] | 15.20 ± 8.87 [ab] | 8.00 ± 7.38 [a] |
| 3 | 23.40 ± 4.82 [a] | 14.10 ± 6.30 [a] | 28.40 ± 6.69 [c] | 17.60 ± 10.73 [a] |
| 4 | 32.60 ± 7.36 [b] | 34.40 ± 15.77 [bc] | 9.20 ± 3.89 [a] | 10.40 ± 6.69 [a] |
| **Osmolarity *(mosmol/L)*** | | | | |
| 1 | 282.20 ± 16.57 [b] | 178.20 ± 67.37 [a] | 261.20 ± 14.9 [a] | 197.00 ± 90.69 [a] |
| 2 | 290.40 ± 14.32 [b] | 260.80 ± 91.13 [a] | 237.80 ± 86.23 [a] | 210.00 ± 105.59 [a] |
| 3 | 212.60 ± 66.08 [a] | 219.80 ± 82.29 [a] | 245.60 ± 14.29 [a] | 259.00 ± 93.05 [a] |
| 4 | 216.40 ± 65.66 [a] | 240.40 ± 101.93 [a] | 280.80 ± 14.65 [a] | 229.80 ± 77.92 [a] |
| **$Na^+$ *(mEq/L)*** | | | | |
| 1 | 118.12 ± 9.87 [a] | 119.26 ± 9.09 [a] | 127.70 ± 9.83 [a] | 132.06 ± 23.58 [a] |
| 2 | 107.12 ± 16.18 [a] | 129.64 ± 8.50 [a] | 123.80 ± 3.54 [a] | 122.30 ± 24.90 [a] |
| 3 | 120.60 ± 11.61 [a] | 121.90 ± 13.47 [a] | 129.78 ± 11.68 [a] | 127.62 ± 16.98 [a] |
| 4 | 116.10 ± 11.31 [a] | 120.56 ± 12.03 [a] | 118.76 ± 6.42 [a] | 104.66 ± 12.97 [a] |
| **$K^+$ *(mEq/L)*** | | | | |
| 1 | 5.90 ± 0.75 [b] | 5.74 ± 0.94 [a] | 5.56 ± 1.38 [a] | 6.90 ± 3.43 [a] |
| 2 | 4.65 ± 0.98 [a] | 5.26 ± 0.47 [a] | 5.58 ± 0.61 [a] | 5.40 ± 3.57 [a] |
| 3 | 5.12 ± 0.72 [ab] | 6.06 ± 0.77 [a] | 4.54 ± 0.39 [a] | 5.62 ± 2.67 [a] |
| 4 | 4.92 ± 0.79 [ab] | 4.74 ± 1.27 [a] | 4.44 ± 0.82 [a] | 5.32 ± 2.98 [a] |
| **$Cl^-$ *(mEq/L)*** | | | | |
| 1 | 100.54 ± 2.29 [a] | 97.46 ± 4.34 [a] | 98.94 ± 10.16 [a] | 105.16 ± 10.16 [a] |
| 2 | 98.66 ± 5.41 [a] | 102.84 ± 7.89 [a] | 101.44 ± 6.54 [a] | 100.30 ± 15.42 [a] |
| 3 | 101.18 ± 3.56 [a] | 100.14 ± 8.49 [a] | 104.60 ± 4.58 [a] | 102.24 ± 15.75 [a] |
| 4 | 99.64 ± 2.93 [a] | 102.26 ± 5.71 [a] | 102.16 ± 8.56 [a] | 101.20 ± 14.62 [a] |

*Values *(*mean ±SD, 5 replicates*)* in the same column followed by different superscripts indicate significant difference *(*$P<0.05$, ANOVA, Duncan*)*, Ct20C = control fish at low water temperature, LD30 = 30% fish died at 4-day, LD50 = 50% fish died at 4-day, LD90 = 90% fish died at 4-day

**Table 4.** Blood parameter of chronic stress in hybrid catfish caused by *A. hydrophila* infection raised in high water temperature *(29.5 ± 0.5°C)*.

| Time *(day)* | Ct30C | LD30 | LD50 | LD90 |
|---|---|---|---|---|
| **Blood clotting time *(s)*** | | | | |
| 1 | 111.40 ± 8.44 [b] | 86.20 ± 30.26 [a] | 115.60 ± 16.07 [b] | 84.80 ± 20.99 [ab] |
| 2 | 90.80 ± 7.69 [ab] | 90.20 ± 7.01 [a] | 97.80 ± 9.78 [ab] | 73.60 ± 20.30 [a] |
| 3 | 87.20 ± 25.46 [a] | 97.80 ± 3.70 [a] | 84.80 ± 16.96 [a] | 101.20 ± 7.79 [b] |
| 4 | 76.20 ± 16.45 [a] | 101.80 ± 8.70 [a] | 85.20 ± 9.17 [a] | 97.60 ± 6.26 [b] |
| **Cortisol *(ng/mL)*** | | | | |
| 1 | 44.86 ± 18.91 [a] | 150.16 ± 16.17 [b] | 76.97 ± 7.01 [c] | 78.97 ± 14.73 [b] |
| 2 | 28.67 ± 12.24 [a] | 31.23 ± 17.11 [a] | 32.43 ± 7.55 [b] | 45.66 ± 44.43 [ab] |
| 3 | 35.81 ± 14.39 [a] | 25.10 ± 7.32 [a] | 23.15 ± 8.39 [ab] | 21.12 ± 9.04 [a] |
| 4 | 25.47 ± 6.21 [a] | 13.63 ± 16.43 [a] | 18.55 ± 6.89 [a] | 44.56 ± 17.40 [ab] |
| **Glucose *(mg%)*** | | | | |
| 1 | 38.80 ± 6.30 [a] | 51.00 ± 8.97 [b] | 69.40 ± 14.38 [c] | 51.8 0± 14.07 [c] |
| 2 | 94.80 ± 9.20 [b] | 66.40 ± 22.55 [b] | 23.80 ± 7.32 [a] | 33.20 ± 7.46 [b] |
| 3 | 41.20 ± 6.87 [a] | 22.40 ± 8.17 [a] | 45.40 ± 21.59 [b] | 20.80 ± 7.72 [ab] |
| 4 | 36.60 ± 8.67 [a] | 14.80 ± 5.63 [a] | 36.80 ± 8.40 [ab] | 18.20 ± 5.35 [a] |
| **Osmolarity *(mosmol/L)*** | | | | |
| 1 | 312.40 ± 28.93 [b] | 296.20 ± 13.60 [b] | 249.40 ± 11.78 [a] | 275.80 ± 15.38 [b] |
| 2 | 285.60 ± 5.94 [a] | 320.60 ± 21.03 [bc] | 286.80 ± 12.59 [b] | 310.20 ± 11.05 [c] |
| 3 | 266.40 ± 17.88 [a] | 247.60 ± 7.66 [a] | 291.80 ± 11.9 [bc] | 313.40 ± 9.78 [c] |
| 4 | 265.40 ± 11.88 [a] | 329.60 ± 25.47 [c] | 310.20 ± 18.61 [c] | 250.40 ± 19.24 [a] |
| **$Na^+$ *(mEq/L)*** | | | | |
| 1 | 127.04 ± 2.86 [b] | 126.32 ± 1.02 [b] | 126.46 ± 2.79 [a] | 129.96 ± 3.36 [a] |
| 2 | 123.48 ± 2.44 [a] | 119.66 ± 9.44 [ab] | 127.68 ± 3.99 [a] | 131.20 ± 1.90 [a] |
| 3 | 121.90 ± 1.81 [a] | 120.46 ± 1.74 [ab] | 126.04 ± 0.81 [a] | 128.32 ± 2.00 [a] |
| 4 | 123.18 ± 1.61 [a] | 112.58 ± 8.01 [a] | 127.72 ± 1.30 [a] | 130.08 ± 2.58 [a] |
| **$K^+$ *(mEq/L)*** | | | | |
| 1 | 4.80 ± 0.38 [a] | 4.62 ± 0.37 [a] | 5.36 ± 0.63 [a] | 4.56 ± 0.41 [a] |
| 2 | 4.68 ± 0.87 [a] | 4.72 ± 0.69 [a] | 4.98 ± 1.05 [a] | 4.74 ± 0.28 [a] |
| 3 | 5.58 ± 0.17 [b] | 4.32 ± 0.66 [a] | 4.26 ± 0.30 [a] | 4.88 ± 1.21 [a] |
| 4 | 4.84 ± 0.24 [a] | 4.28 ± 0.22 [a] | 4.86 ± 0.92 [a] | 5.06 ± 0.37 [a] |
| **$Cl^-$ *(mEq/L)*** | | | | |
| 1 | 94.24 ± 11.69 [ab] | 98.24 ± 4.61 [b] | 101.10 ± 2.66 [ab] | 103.42 ± 7.55 [b] |
| 2 | 87.88 ± 7.40 [a] | 101.90 ± 4.14 [b] | 102.40 ± 2.15 [b] | 100.26 ± 1.34 [ab] |
| 3 | 98.42 ± 3.03 [b] | 95.48 ± 2.78 [b] | 102.12 ± 2.33 [b] | 97.32 ± 1.99 [a] |
| 4 | 100.02 ± 3.06 [b] | 83.82 ± 10.59 [a] | 97.86 ± 3.74 [a] | 103.20 ± 1.90 [b] |

***** Values *(*mean ±SD, 5 replicates*)* in the same column followed by different superscripts indicate significant difference *(*P<0.05, ANOVA, Duncan*)*, Ct30C = control fish at high water temperature, LD30 = 30% fish died at 4-day, LD50 = 50% fish died at 4-day, LD90 = 90% fish died at 4-day

**Electrolytes (Na$^+$, K$^+$ and Cl$^-$)**

Fish in the control group showed stable levels of Na$^+$ and Cl$^-$. This might be due to the function and constant concentration of cortisol. Even though electrolytes are regulated by cortisol, the permeability and chloride cell of fish gills might not be affected (Ruane et al., 2001).

In the acute group, hybrid catfish exhibited decreased K$^+$. This result is the same as Chen et al. (2004) who found low K$^+$ in vibriosis Nile tilapia. Meyers et al. (2002) also reported that K$^+$ was reduced in viral hemorrhagic septicemia in rainbow trout.

Fish from the chronic group showed reduction of Na$^+$ and Cl$^-$. The downward level might be because of the cortisol effect and the catfish being in a stressed situation. This study is agreement with Al-Dohail et al. (2011) who reported the reduction of Cl$^-$ in bacterial infected African catfish. Chen et al. (2004) also demonstrated low Cl$^-$ of streptococcosis in Nile tilapia. Since fish are hyperosmotic to the environment and produce high quantities of urine, a reduction of urine flow rates must be activated in fish to save salt in body fluids.

Cortisol stimulates ion regulatory changes during stress (Peres et al., 2012). The decreased Na$^+$, K$^+$ and Cl$^-$ in the infected group in this study may be assumed to have increased the outflux of these ions through the fish gills. To compensate for the constant plasma electrolytes, catfish may accumulate Na$^+$ and Cl$^-$ by active absorption from the environmental water which occurs at the branchial cells (Meyer et al., 2002).

## Conclusion

Even though the catfish in the control group were IP with saline water, the fish were not stressed. Catfish under infection stress display significant changes in blood parameters that can be used as stress indicators. The obtained values are variable within and among groups. These infected fish cannot be distinguished from non-infected fish except for their lethargic and anorexic performance. This study suggests that fish blood is a sensitive method for detecting the presence of bacterial stressors. The use of blood glucose as an indicator of bacterial stress in hybrid catfish was a good index for this research. However, a single blood parameter is not adequate for chronic and acute bacterial stress detection. Information from aquaculture management should be obtained.

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